detection for distinguishing labelled compounds. Preferably the improved assays display more than one of these features and preferably they display all of these features. The present invention seeks to provide novel reagents and methods for performing such an assay.

Summary of Invention

According to a first aspect of the present invention, there is provided a compound of Formula I:

R-L-S

(I)

wherein R is a fluorescent dye molecule;

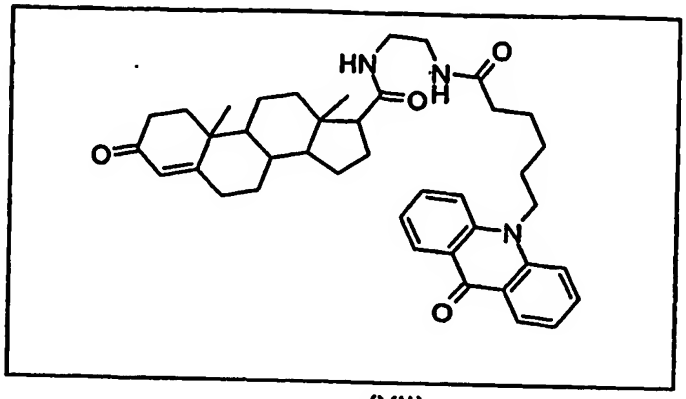
L is an optional linkage group containing one or more atoms comprising hydrocarbon chains which may also contain other atoms such as N, O and S; and

S is a molecule comprising a substrate group of the enzyme aromatase

characterised in that the fluorescence signal of said compound changes in respect of fluorescence lifetime when the compound is acted upon by an enzyme with aromatase activity.

A range of fluorescent labels are commercially available which could be used as a fluorescent reporter moiety R in accordance with the present invention. Examples include, but are not limited to, oxazine (e.g. MR 121, JA 242, JA 243) and rhodamine derivatives (e.g. JA 165, JA 167, JA 169) as described in WO 02/081509. Other examples (as described in WO 02/056670) include, but are not limited to Cy5, Cy5.5 and Cy7 (Amersham); merocyanine (Few Chemicals), IRD41 and IRD700 (Licor); NIR-1 and IC5-OSu (Dojindo); Alexa fluor 660 & Alexa fluor 680 (Molecular Probes); LaJolla Blue (Diatron); FAR-Blue, FAR-Green One & FAR-Green Two (Innosense); ADS 790-NS and ADS

In a preferred embodiment of the first aspect, there is provided a compound of Formula XII



(XII)

In a second aspect of the present invention, there is provided a method for measuring aromatase activity in a sample, the method comprising the steps of:

i) measuring the fluorescence lifetime of a compound according to any preceding claim prior to adding it to said sample;

ii) adding said compound to said sample under conditions which favour aromatase activity, and

iii) measuring a change in fluorescence lifetime of said compound following step ii);

wherein said change in fluorescence lifetime can be used to determine aromatase activity.

Suitably, the sample is selected from the group consisting of extract, cell, tissue and organism. The cell or organism may be naturally occurring or may be a recombinant cell or organism which has been genetically engineered to over-express a particular protein, such as aromatase.

Suitably, conventional detection methods can be employed to measure fluorescence intensity and/or the lifetime of the label. These methods include instruments using photo-multiplier tubes as detection devices. Several approaches are possible using these methods; e.g.

i) methods based upon time correlated single photon counting (cf. Principles of Fluorescence Spectroscopy, (Chapter4) ed. J R Lakowicz, Second Edition, 1999, Kluwer/Academic Press)

ii) methods based upon frequency domain/phase modulation (cf. Principles of Fluorescence Spectroscopy, (Chapter5) ed. J R Lakowicz, Second Edition, 1999, Kluwer/Academic Press)

iii) methods based upon time gating (cf. Sanders et al., (1995) Analytical Biochemistry, 227 (2), 302-308).

Measurement of fluorescent intensity may be performed by means of a charge coupled device (CCD) imager, such as a scanning imager or an area imager, to image all of the wells of a multiwell plate. The LEADseeker™ (Amersham Biosciences, UK) system features a CCD camera allowing imaging of high density microtitre plates in a single pass. Imaging is quantitative and rapid, and instrumentation suitable for imaging applications can now simultaneously image the whole of a multiwell plate.

According to a fifth aspect of the present invention, there is provided a method for measuring the distribution of a compound as hereinbefore described within a tissue, wherein the compound is capable of being taken up by a living cell within the tissue, the method comprising the steps of:

i) measuring the fluorescence lifetime of the compound in a cell-free environment or a parental host cell;

ii) adding the compound to one or more cells or a cell engineered to over-express aromatase, and

iii) measuring the fluorescence lifetime of the compound following step ii);

wherein a change in fluorescence lifetime indicates aromatase activity and can be used to determine the distribution of the